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PROLONGED EXPOSURE OF ANIMALS TO PRESSURIZED  
NORMAL AND SYNTHETIC ATMOSPHERES

by

Robert D. Workman,  
CDR, MC, USN,

George F. Bond,  
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Walter F. Mazzone,  
CDR, MC, USN,

Bureau of Medicine and Surgery, Navy Department  
Research Project MR005.14-3100-3.02

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Bureau of Medicine and Surgery, Navy Department  
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**Approved by:**

**Walter R. Miles, Ph.D.  
Scientific Director**

**Released by:**

**George F. Bond  
CDR, MC, USN  
Officer-in-Charge**

## **SUMMARY PAGE**

### **THE PROBLEM**

To expose experimental animals, including primates, to natural and synthetic atmospheres under conditions of pressure equivalent to 200 feet of sea water, for periods up to two weeks; and subsequently to conduct adequate decompression of these animals before their removal from the experimental chamber.

### **FINDINGS**

Results of this study confirm that survival of these experimental animals in a selected atmosphere of helium and oxygen can be predicted. Although these animals cannot survive a high pressure of air for more than thirty-five hours, an equivalent exposure to a selected synthetic breathing gas mixture is tolerated for a period of two weeks without physiological deterioration.

### **APPLICATION**

The information gained in this investigation is of value in planning extension of the experimental design to include human subjects. Final application of the data derived from human experiments may be utilized in planning atmosphere and environmental control for operational exercises involving prolonged deep diving procedures; extensive underwater construction concepts; deep salvage operations; or in oceanographic and marine biological research on the continental shelves of the world.

### **ADMINISTRATIVE INFORMATION**

This investigation was undertaken as a part of Bureau of Medicine and Surgery Research Task MR005.14-3100, under Subtask (3)—Effect of Prolonged Exposure to High Ambient Pressures of Synthetic Gas Mixtures. The present report is No. 2 on this Subtask and was approved on 26 January 1962.

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## **ABSTRACT**

In order to explore the problem of mammalian survival under high ambient pressures of synthetic atmospheres, it was elected to expose several species of experimental animals to a selected artificial atmosphere at a simulated depth of 200 feet of sea water for a period of fourteen days. In a progressive series of experiments, colonies of rats, guinea pigs, and finally, squirrel monkeys, were so exposed. Temperature, humidity, carbon dioxide and ammonia levels were controlled with increasing success, as the experiments proceeded. Feeding and water supplies were automatically supplied to the isolated experimental animals. Although early deficiencies in experimental design posed problems, all animals in the series survived with no demonstrable physiological lesions in excess of endemic findings of control animals. In the final experiment, in which primates were utilized, there was no evidence of immediate or delayed adverse physiological effects.

The results of this series of animal exposures to high pressures of synthetic atmospheres indicate that: (1) In the case of the experimental animals employed, i.e., rats, guinea pigs, and squirrel monkeys, survival in a nitrogen-free atmosphere can be predicted with assurance; and (2) Although these animals can survive only brief periods in an environment of compressed air at 200 feet simulated depth, exposure to an equivalent total pressure of a selected synthetic mixture is tolerated for a period of fourteen days without physiological deterioration.

In a second series of experiments described in this report, an effort was made to establish limits of adequate decompression for a completely saturated, equilibrated, large experimental animal. For this purpose, goats were selected as ideal animal subjects. After equilibration at exposure depth of two hundred feet equivalent of sea water, random-mixed pairs of animals were decompressed on ratios which varied with respect to absolute bottom-pressure versus absolute decompression stages ambient. From these experiments, it is believed that a safe decompression ratio for gas-saturated humans can be established. It is finally concluded that human experiments may now be pursued with safety, on an increasing scale.

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# PROLONGED EXPOSURE OF ANIMALS TO PRESSURIZED NORMAL, AND ARTIFICIAL ATMOSPHERES

## INTRODUCTION

This report presents the results of a series of prolonged exposures of various mammals to normal air and to synthetic atmospheres at a pressure of 200 feet of sea water. The experimental objective was to provide a respirable atmosphere adequate to maintain normal physiologic functions in the exposed animals for 12-14 days, then to reduce the pressure in suitable stages so that decompression sickness might not occur.

Provision of a suitable atmosphere for man would make possible long-term exposure for work in underwater stations, and return to the surface following work periods in stations at intermediate depths selected to avoid decompression sickness.

Several authors (1, 2, 3, 4, 5, 6) have presented evidence that increased oxygen tension, rather than barometric pressure itself, is responsible for the morbidity and mortality occurring under conditions of prolonged exposure to high pressure air. Smith et al, (6), in 1932 found that albino rats exposed to optimal conditions of temperature and humidity on air with a pressure of four atmospheres (99 feet equivalent sea water depth) developed some cases of oxygen poisoning on the third day, and 13% mortality occurred on the fourth day. However, animals that survived on air for 40 days at this pressure level were resistant to oxygen toxicity on a subsequent exposure. All rats dying during exposure to high oxygen pressure (635 mm Hg) showed evidence of hyperemia and edema of lungs.

Barach (7) reported in 1935 that mice breathing an atmosphere of 79% helium and 21% oxygen at one atmosphere pressure for ten weeks suffered no harmful effects. This challenging experimental finding has not had the influence on respiratory research that might have been expected. To our knowledge, no subsequent reports have been published concerning prolonged exposure of animals to helium-oxygen mixtures at high pres-

ures. There is, however, one such report of exposure of men for 12 hour periods, at pressures of 2-2.6 atmospheres, by Duffner and Snider, in 1950, (11). In our experiments, with helium-oxygen mixtures, the oxygen tension was maintained equivalent to air at one atmosphere, (160 mm Hg) to avoid risk of pulmonary damage, and a variety of mammals other than man served as subjects.

Decompression studies, following exposures to increased pressures, were carried out on goats, since, of all laboratory animals generally studied, goats compare most closely to man for decompression requirements. A considerable body of information is available concerning a wide range of pressure exposure for goats to air atmospheres (8, 9, 10). As mentioned, human exposures to helium-oxygen atmospheres for periods of 12 hours have been carried out previously and decompression requirements determined for these subject groups (11). Our overall objective was to expand this research and determine the viability of various animals in long-continued exposure to simulated depths of 200 feet of water, while breathing synthetic gas mixtures.

## GENERAL METHODS AND PROCEDURE

In our series of experiments albino rats were first exposed to normal air at a pressure of seven atmospheres absolute to establish mortality and histopathological change under this condition. In the second experiment, albino rats and guinea pigs were exposed to a pressure of seven atmospheres absolute, breathing a nitrogen-oxygen atmosphere with  $pO_2$  maintained at 160 mm Hg to determine whether pulmonary changes seen on the air exposure might in this manner be averted. In the third experiment, a similar exposure at seven atmospheres absolute was carried out with  $pO_2$  maintained at 160 mm Hg, but with helium substituted

for nitrogen as the inert component in the synthetic gas mixture breathed by the animals.

In the fourth experiment, squirrel monkeys were substituted for rats and guinea pigs in the helium-oxygen exposure to determine whether primates would respond in a more sensitive manner under these conditions.

In the fifth experiment, three pairs of goats were exposed successively to a helium-oxygen atmosphere in which the  $pO_2$  was maintained at 160 mm Hg. Following a 72 hour exposure to insure equilibration of tissues to the synthetic atmosphere, the animals were surfaced in stages with stops of 36 hours at 84 and 26 feet respectively to determine whether decompression sickness could be avoided. In each of these experiments, decompression was accomplished in timed stages. A section devoted to the study of decompression is presented later in this paper.

Details of the above outlined experiments are described in the following sections of this report, (a) through (d), including the specific method used for animal maintenance and for atmosphere control.

#### (a.) Chronic Exposure of Albino Rats to High Pressure

##### Method:

In this representative experiment of our series, twenty-four young male rats (Wistar strain) were segregated, two in a cage, with ample supplies of food and water. They were allowed to acclimatize in a standard U. S. Navy recompression chamber, of 350 cubic feet capacity, for a period of four days and then were subjected to pressurized air (in a transition period of five minutes) to a simulated depth of 200 feet of sea water. The chamber was ventilated at a constant pressure for four to six minutes each hour

to avoid excess carbon dioxide accumulation in the atmosphere. Every half-hour observations were made of chamber temperature, pressure, relative humidity, and the condition of the animals. Death of animals was ascertained by absence of visible respiratory movements. When all animals appeared to be dead, the chamber was "brought to the surface" within a period of seven minutes, and deaths were verified by observations of body temperature and absence of respiration. Three animals lived to within 30 minutes of the time the chamber was surfaced and these were autopsied immediately.

Temperature as recorded by thermograph was relatively constant throughout the experimental period, after the initial rise due to compression, and ranged from 76°F to 80°F. Relative humidity determined by wet-dry bulb thermometer averaged 96%.

##### Results:

The animals appeared lethargic after 15 hours at 200 feet. At this time hyperpnea was observed in most animals, as well as cyanosis of the ears and nose. The first death was recorded at 28 hours of pressure exposure; 50% mortality was attained at 30 hours; and all animals were dead at the conclusion of the 35th hour. Gross examination of the thoracic cavities of three longest surviving animals revealed numerous petechiae with marked diffuse hyperemia, and large, well delineated areas of necrotizing pneumonitis. In addition, bilateral pleural effusions were found. Each side of the chest was observed to contain clear, straw colored fluid in excess of one ml. The heart and great vessels were observed to be grossly normal. No evidence of froth or air bubbles was observed in them. Histopathological studies revealed interstitial hemorrhage in the myocardium, intra-alveolar hemorrhage, pneumonia, edema of the lungs (see Figure 1) and interstitial hemorrhage of kidneys.

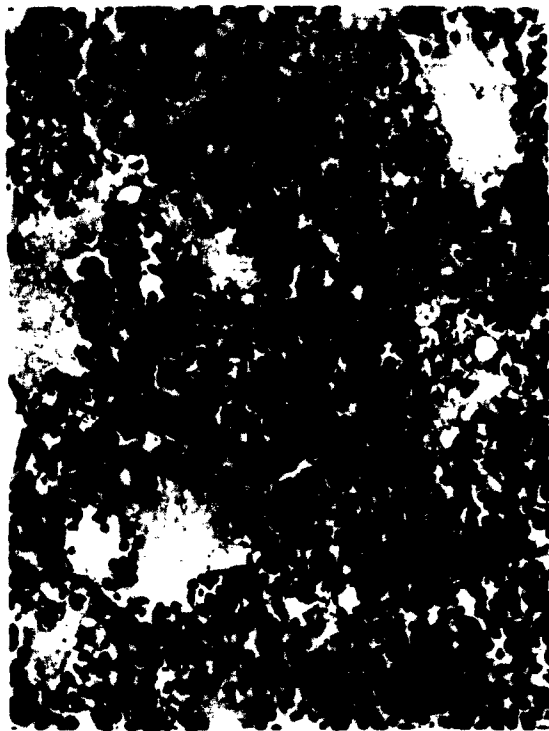


Figure 1—Rat Lung: Animal was exposed to compressed air (200 feet) until death supervened (approximately 34.5 hours). Note excessive pulmonary edema and pneumonitis.

H&E x 350

(b.) Chronic Exposure of Albino Rats and Guinea Pigs to High Pressure  
97% Nitrogen — 3% Oxygen.

**Method:**

As representative of a series using a reduced  $O_2$ , twenty-four adult male rats (Wistar strain) and four guinea pigs were exposed to a simulated depth of 200 feet of sea water in a pressure chamber while breathing a 97% nitrogen — 3% oxygen mixture for 14 days. At a total pressure of 7 atmospheres absolute, or 5320 mm Hg, the oxygen content of the gas in the chamber remained at 160 mm Hg, or 3% of the total pressure. This becomes 21% physiologically effective oxygen at this depth (3% x 7 atmospheres). Carbon dioxide produced by the animals was absorbed by means of 40 pounds of granular soda lime spread in thin layers in shallow trays. Twenty pounds of silica gel was used for water vapor absorption. Two electric fans were operated continuously to provide circulation of the

chamber atmosphere. Self-maintaining cages provided supplies of food and water for a period of one week. Cage-litter mixed 10:1 with boric acid powder was used in screen-covered litter trays beneath the cages to reduce ammonia formation from excreta. On the 8th day, soda lime, litter, food and water were replenished by a diver who entered the inner chamber after equalizing the outer chamber with nitrogen from pressurized cylinders. Carbon dioxide and oxygen content of the chamber atmosphere were analyzed by Scholander Micro-Gas Analyzer and Beckman Model-C Oxygen Analyzer. Oxygen was added to the chamber from pressurized cylinders as required to maintain concentration at 3% (160 mm Hg). Nitrogen was added to the chamber from pressurized cylinders to maintain pressure constant at 200 feet.

Decompression was carried out over a 24-hour period with stops at 5, 4, 3, and 2 atmospheres absolute pressure,—see graph, Figure 2.

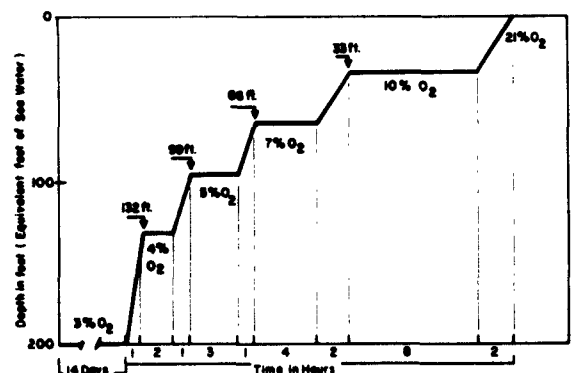


Figure 2—Decompression of rats and guinea pigs from 14 days exposure on 97% helium and 3% oxygen at atmospheric pressure.

Oxygen concentration was maintained at an effective pressure of 21% (160 mm Hg) during the decompression period.

Animals were sacrificed immediately after the chamber "surfaced," and at 10 and 30 days after surfacing. Blood was withdrawn for biochemical determinations from the left ventricles of sacrificed animals while the heart was still actively contracting. These determinations included whole blood pH,  $CO_2$  content, oxygen content, blood urea nitrogen (BUN), blood sugar, hemoglobin, and plasma



sodium and potassium. Methods used for these analyses are described in experiment (d), Page 7.

#### Results:

One rat died on the third day from unknown cause. All other animals survived the 14 day duration of the experiment. Analyses of carbon dioxide in the chamber atmosphere showed an effective concentration of less than 0.5% during the exposure period. Oxygen concentration was maintained at  $160 \pm 10$  mm Hg. Blood biochemical studies, Table I, upon surfacing and ten days after surfacing, showed no significant difference from control animals. Histopathological studies on six animals (four rats and two guinea pigs), (Figures 3, 4, 5) sacrificed upon surfacing, showed four to have focal pulmonary atelectasis, three with focal pneumonia, (Figure 3) and three with adrenal changes characterized by decreased lipid material in cortex and zona fasciculata. One of four animals sacrificed ten days after surfacing showed focal bronchopneumonia, (Figure 4). No histopathological abnormalities were demonstrated in three rats sacrificed 30 days after surfacing.

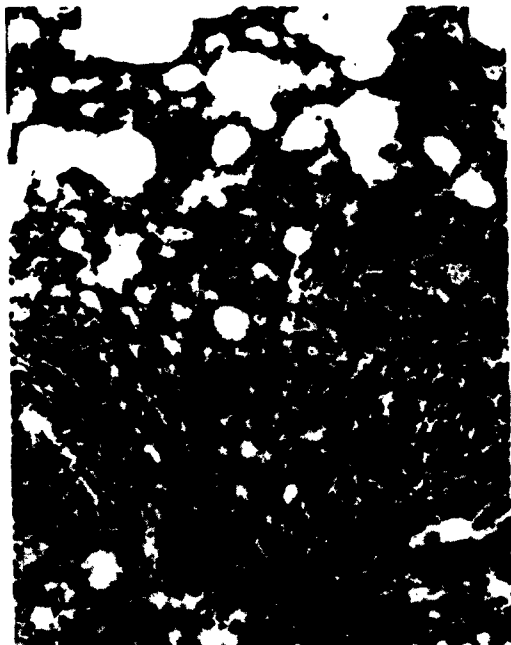


Figure 3—Rat Lung: Animal was exposed in 97% N<sub>2</sub>—3% O<sub>2</sub> at 200 feet for two weeks and sacrificed two hours after surfacing. Note focal zones of atelectasis and pneumonitis.

H&E x 130

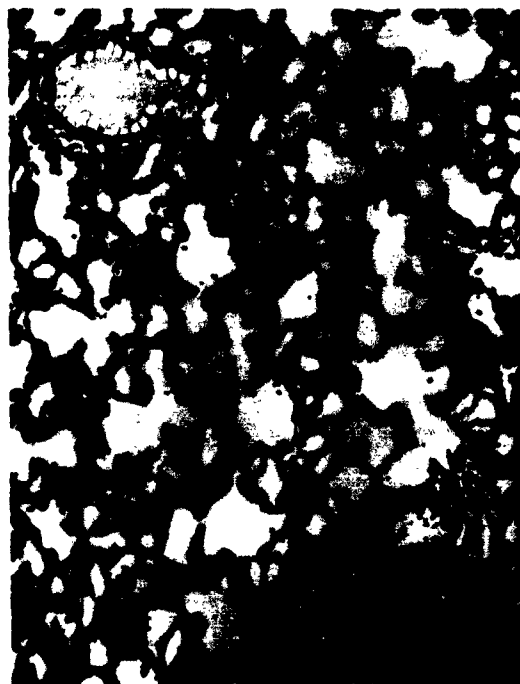


Figure 4—Rat Lung: Animal was exposed in 97% N<sub>2</sub>—3% O<sub>2</sub> at 200 feet for two weeks and sacrificed ten days after surfacing. The alveolar walls remained thickened by the endemic pneumonitis but the atelectasis is diminished.

H&E x 130



Figure 5—Rat Brain (Hippocampus): Animal was exposed in 97% N<sub>2</sub>—3% O<sub>2</sub> at 200 feet for two weeks and sacrificed two hours after surfacing. Note loss of neurons (arrows) from a hippocampal band.

Nissl x 90

Ten rats lost an average of 55 grams weight during the exposure period, while four rats showed no weight change. Six rats gained an average of 11 grams in weight. The four guinea pigs lost an average of 54 grams in weight. In this connection, it is probably significant that aspergillus mold appeared on the commercial rat biscuits at the end of the first week of exposure, after which the rats did not feed as actively as before. Furthermore, difficulty was encountered with the guinea pig feeders not dropping feed effectively so that adequate supplies of feed were not always available. These factors may have influenced uniform weight loss in the animals. Chamber temperature varied between 78°F, averaging 85°F. Relative humidity determined by dry-wet bulb readings ranged from 72% to 96%, averaging 87%.

The rats appeared sluggish and several animals dragged their hind legs intermittently during the exposure, (Figure 5). The guinea pigs were normally active and alert throughout.

**Table I—Blood Studies on Animals Exposed to 97% Nitrogen —3% Oxygen at Seven Atmospheres for Fourteen Days**

	MEAN VALUES					
	Immediate		10 Days Survival		Controls	
	Rats G. Pigs	Rats G. Pigs	Rats G. Pigs	Rats G. Pigs	Rats G. Pigs	Rats G. Pigs
pH	7.37	7.41	7.39	7.41	7.38	7.40
CO <sub>2</sub> Content (Vol. %)	49.2	52.1	48.6	51.8	50.8	52.5
O <sub>2</sub> Content (Vol. %)	11.6	12.4	11.8	12.4	12.1	12.8
Blood Sugar (mgm %)	134.	141	96	115	105	115
BUN (Blood Urea Nitrogen) (mgm %)	9.7	10.5	11.3	14.1	11.0	10.4
Hemoglobin (gm %)	14.9	14.7	14.4	15.1	14.8	15.4
Plasma Na (MEq/L)	152	150	148	147	148	152
Plasma (MEq/L)	5.3	7.5	5.6	7.5	5.8	6.0

N=4   N=2   N=4   N=2   N=4   N=4

(c.) Chronic Exposure of Albino Rats to a High Pressure of 97% Helium—3% Oxygen

In view of a mortality of 100% attained in the 35th hour of compression when albino rats were exposed to air at seven atmospheres pressure, and focal pulmonary atelectasis and pneumonia when exposed to

normal pO<sub>2</sub> but high pN<sub>2</sub> in experiment (b), an attempt was made to provide a synthetic atmosphere which might make such a pressure exposure possible without significant morbidity and mortality in the experimental animals.

Helium, being a less dense gas than nitrogen, and without apparent narcotic effect in the range of observed pressure exposure (15), should make possible normal alveolar ventilation at seven atmospheres pressure, if we are to assume that inadequate ventilation is a factor in the mortality of animals when air is breathed at this pressure. In addition, the capacity of maintaining pO<sub>2</sub> of the mixture at a value equivalent to that in air at one atmosphere (160 ± 10 mm Hg) should eliminate the possibility of pulmonary damage resulting from long exposures to high pO<sub>2</sub>.

#### Method:

Twenty-four male rats (Wistar strain) ranging from 280-440 grams in weight were placed four to a cage with ample supplies of food and water for eight days. These cages were placed in the outer compartment of our compression chamber to acclimatize to this environment for four days. At the end of this period, the inner chamber was purged with helium and the air mixture was exhausted through a low drain valve until the oxygen content sampled 3% on a Beckman Model-C Oxygen Analyzer, calibrated with helium-oxygen mixtures analyzed on the Scholander Micro-Gas Analyzer. Pressure was then built up to 16 feet simulated depth by addition of helium and oxygen until the oxygen analysis of sampled chamber atmosphere was 14% (160 mm Hg at this pressure). The outer lock containing the animals was then purged with an 80% helium—20% oxygen mixture before pressurization to 16 feet on 100% helium. Upon equalization of inner and outer chamber locks, the animals were taken into the inner lock, which was then pressurized to 200 feet with 100% helium. Oxygen sampled from the inner chamber lock analyzed 3% on the Beckman Oxygen Analyzer, the equivalent of 160 mm Hg at seven atmospheres pressure. These

measures insured removal of the chamber air and its replacement with the final helium-oxygen mixture desired.

Carbon dioxide absorption was accomplished by means of 46 pounds of Baralyme placed on screens high and low in the chamber. Water vapor was absorbed by 25 pounds of activated alumina in pans on the deck of the chamber. Two electric fans were operated continuously to circulate gas in the chamber and insure thorough mixing of gas added from cylinders. Commercial animal litter mixed 10:1 with boric acid powder was used in litter trays to suppress ammonia formation from excreta.

The chamber atmosphere was sampled every 6-8 hours by withdrawing gas from a copper tube, placed at cage level between the rows of cages, into an evacuated rubber anesthesia gas bag. Oxygen was analyzed by the Beckman Model-C Oxygen Analyzer calibrated with helium-oxygen mixtures previously analyzed by Scholander Micro-Gas Analyzer. The latter instrument was also used to analyze oxygen and carbon dioxide content of the chamber. Ammonia concentration was monitored by Draeger analysis tubes. Oxygen was added to the chamber from cylinders via a manifold to maintain a  $pO_2$  of 160 mm Hg. Pressure was maintained at 200 feet by addition of 100% helium to the chamber, as required.

Replenishment of water, food, Baralyme, and cage litter trays was carried out on the eighth day of exposure by entering the outer lock pressurized with helium after initial purge with 80% helium—20% oxygen mixture. The diver was breathing a mixture of 80% helium—20% oxygen from a mask, supplied by a demand valve attached to the gas supply cylinder in the chamber lock.

Exposure of animals continued for fourteen days at seven atmospheres. Conservative decompression was carried out exactly as described in Section (b), page 3, (see Figure 1), to avoid risk of superimposing decompression sickness upon effects of pressure exposure. An oxygen pressure of 160 mm Hg was maintained. This was accomplished by slowly raising this value

just before leaving one level so that it would equal 160 mm Hg upon arriving at the next decompression stop.

Some animals were sacrificed immediately following arrival of the chamber at one atmosphere and others at 8, 14, 30, and 200 days after surfacing. Blood for biochemical determinations was drawn from the left ventricle of animals sacrificed while the heart was still actively contracting. Methods used for these analyses are described in experiment (d).

### Results:

All animals survived and were normally active during the exposure period. With few exceptions the animals lost weight. The weight loss averaged 46 grams per animal. Three weeks after surfacing, the 10 animals which had not been sacrificed had gained an average of 63 grams. As in the previous experiment, development of *aspergillus* mold on the rat food was noted and may have been a significant factor in the weight loss of the animals.

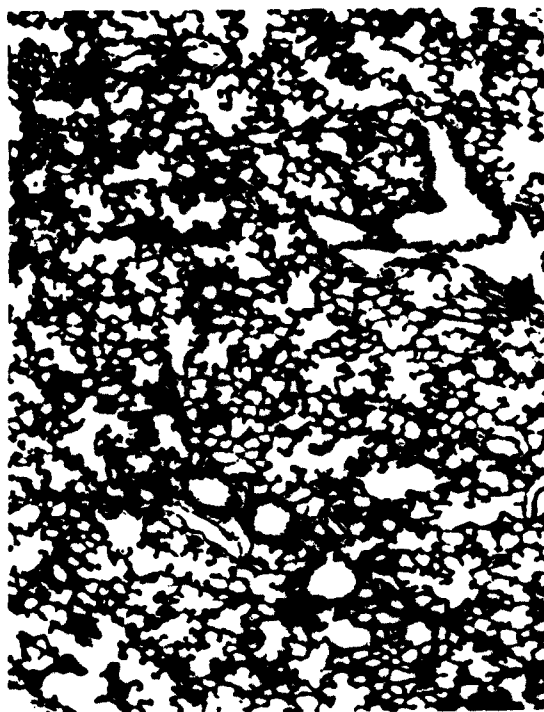
Carbon dioxide analysis of chamber atmosphere showed an effective concentration of less than 0.5% during the exposure. The  $pO_2$  was maintained at  $160 \pm 10$  mm Hg during the exposure period. Temperature in the chamber ranged from 75°F - 83°F. Humidity by wet-bulb thermometers registered between 83 - 94%. No ammonia was detected by Draeger analysis tubes or odor of chamber atmosphere until decreasing chamber pressure during decompression flooded litter trays from drinking water bottles. Blood analysis carried out upon surfacing, and subsequent sacrifices included pH,  $CO_2$  content, hemoglobin, hemotocrit, blood sugar, and blood urea nitrogen. Although our population was small, determinations on exposed animals did not vary importantly from those of control animals. See Table II.

Histological examinations of these animals were performed. No abnormalities were found in the two animals sacrificed immediately after surfacing (Figure 6). All thirteen animals sacrificed from eight days to seven

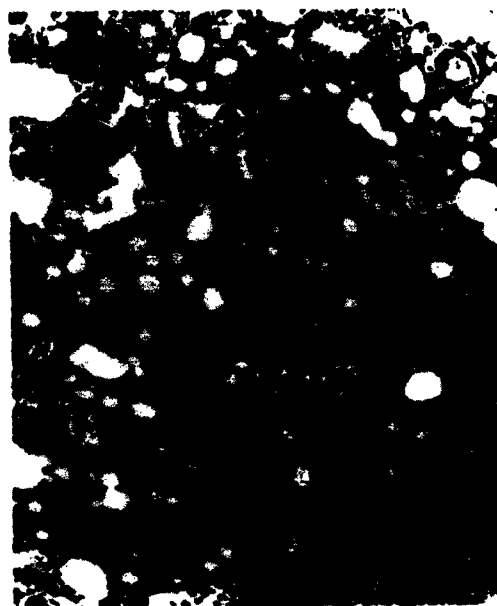
**Table II—Blood Studies on Albino Rats Exposed to 97% Helium—3% Oxygen at Seven Atmospheres for 14 Days (Mean Values)**

	HeO <sub>2</sub> Run—Rats		
	Immediate	1 week	Controls
pH	7.38	7.39	7.39
CO <sub>2</sub> Content (Vol. %)	50.2	50.4	48.8
Hemoglobin (gm %)	14.7	15.2	14.8
Hematocrit	44	45	46
Blood Sugar (mgm %)	96	103	105
Blood Urea Nitrogen (mgm %)	15.0	13.4	12.9
	N=6	4	6

months after surfacing were normal except for two animals in which some fresh intra-alveolar hemorrhage was noted, (Figure 7). Due to the recent nature of this hemorrhage and its proximity to zones of endemic pneumonitis, it was felt that these findings were unrelated to the pressure exposure.



**Figure 6—Rat Lung:** Animal exposed in 97% He—3% O<sub>2</sub> at 200 feet for two weeks and sacrificed immediately after surfacing. Note that the lung is well aerated without evidence of atelectasis.  
H&E x 55



**Figure 7—Rat Lung:** Animal exposed in 97% He—3% O<sub>2</sub> for two weeks and sacrificed ten days after surfacing. Note intense zone of fresh intra-alveolar hemorrhage. This is occasionally noted adjacent to zones of endemic pneumonitis.  
H&E x 90

(d.) Chronic Exposure of Squirrel Monkeys to High Pressure Atmosphere of 97% helium—3% oxygen.

#### Method:

In anticipation of future experimental work in which human subjects might be utilized, it was felt desirable that small primates be exposed to the experimental conditions previously described.

Three squirrel monkeys weighing 425-435 grams were allowed to acclimatize to the environment of our compression chamber for four days in a self-maintained cage. At the end of this time they were pressurized to seven atmospheres, (200 feet equivalent depth of sea water) in the chamber by addition of helium from pressure cylinders. The oxygen concentration at this pressure was equivalent to 21%, though diluted to 3% ( $160 \pm 10$  mm Hg) of the total gas pressure of 5320 mm Hg. At the beginning, no attempt was made to purge the air completely from the chamber. Through frequent additions of helium to maintain chamber pressure, the pN<sub>2</sub> was continuously reduced

during the exposure period. The partial pressure of oxygen was maintained at  $160 \pm 10$  mm Hg during the exposure by the addition of oxygen to the chamber from pressurized cylinders. Carbon dioxide removal was carried out by operation of a Desomatic Carbon Dioxide Removal Unit containing four canisters of soda lime of 9.4 lbs. each. One canister of silica gel in that unit was used to remove water vapor from the chamber atmosphere. An additional 10 lbs. of silica gel was spread in a thin layer in two shallow trays. Two electric fans operated continuously to circulate gas in the chamber and insure mixing of the chamber atmosphere. Oxygen concentration was maintained at  $160 \pm 10$  mm Hg during the exposure. Measurements of 0.1% carbon dioxide concentration (0.7% effective concentration) were obtained after periods of 12 hours without use of the CO<sub>2</sub> removal unit. Operation of the Desomatic Unit for 30 minutes at 70 cfm capacity reduced the sampled atmosphere analysis to 0% CO<sub>2</sub>. Chamber temperature ranged from 82°-90°F with an average of 85°F. The animals ate well and were normally active during the exposure period. Weight was maintained and an average gain of 15 grams per animal occurred during the exposure.

Carbon dioxide content of the chamber atmosphere was analyzed by high level Kiti-gawa carbon dioxide analysis tubes twice daily. Gas samples were withdrawn from the chamber by a sampling tube placed near the animal cage. Oxygen concentration of chamber atmosphere was analyzed with Beckman & Scholander apparatus, exactly as described in the two previous tests.

The monkeys were fed a commercial monkey biscuit ration daily by timed release of a solenoid-activated feed hopper. Additional rations of bananas, white grapes, and apples were placed in the cage at the beginning of the exposure, and on the seventh day, when water bottles, feed and litter were renewed. This housekeeping was performed by a diver breathing 80% helium—20% oxygen, with the outer lock of the chamber pressurized with helium to equalize with the inner lock.

Commercial animal litter mixed 10:0 with boric acid powder was used in the litter tray to decrease ammonia formation by urea-splitting bacteria in excreta. Fifty mgm of veterinary type aureomycin powder was added to each 1000 cc water bottle to limit intestinal infections encountered in monkeys of this kind.

Decompression was begun after the 12th day of exposure by reduction of the chamber pressure from 7 to 3.5 atmospheres (84 feet equivalent depth of sea water). The partial pressure of oxygen was maintained at  $160 \pm 10$  mm Hg by increasing the oxygen concentration from 3% to 6% before reduction of pressure. After a 24 hour stop at 3.5 atmospheres, pressure was decreased to 1.75 atmospheres (26 feet) where the oxygen concentration was maintained at 12%. After 24 hours at 1.75 atmospheres, the oxygen concentration was increased to 17% before the chamber pressure was reduced to one atmosphere. Before one hour had elapsed after reaching the surface, two monkeys were sacrificed. Prior to sacrificing, 12 mgm of pentobarbital was injected peritoneally, the monkeys were weighed, and left heart blood was drawn into a heparinized glycerin-coated syringe under direct visualization of the heart through the opened thoracic cage. The third exposed monkey was maintained in the animal suite for a period of two weeks to observe changes, if any, following recovery in normal air atmosphere. Two control monkeys had been maintained in a similar cage in the outer chamber lock, throughout the experiment, where conditions of heat and humidity were comparable to those experienced by the three monkeys exposed to pressure. One of these controls was sacrificed at the same time as the initial two exposed to pressure, and the other at the end of the two weeks.

The CO<sub>2</sub> content and O<sub>2</sub> content of heparinized anaerobically sampled, whole blood were measured in duplicate within one hour after withdrawal by the manometric method of Van Slyke and Neill (16). The pH determinations were made on the whole blood

at 37.5°C with the Sanz glass electrode and the Metrohm E322 compensator by the method of Gambino and Arends (17). Plasma  $\text{CO}_2$  content was calculated from whole blood  $\text{CO}_2$  by means of the nomogram of Sendroy, Dillon and Van Slyke (18). Plasma  $\text{CO}_2$  tension was calculated by means of the Henderson-Hasselbalch equation from the observations of  $\text{CO}_2$  content, pH, oxygen content and saturation using the  $\text{pK}'$  and solubility factors, corrected to body temperature, of Severinghaus. Values of 6.10 for  $\text{pK}'$  at 37°C and 0.031 for solubility of  $\text{CO}_2$  were used.

Blood urea and urea nitrogen content were measured by the method of Gentzkow (20). Sodium and potassium measurements were done with the Baird flame photometer. Blood chlorides were measured by the method of Schoales and Schoales (21). Blood sugar was measured by Nelson's adaptation of the Somogyi method (22). Carbonic anhydrase determinations were performed by the method of Altschule and Lewis (23).

Histopathological evaluation of the monkeys, as in experiment (c), included all vital organs as well as eyes, gonads, bone marrow and long bones. Radiographs of long bones were done on both experimental and control animals to check the possible existence of bony lesions attributable to decompression effects.

#### Results:

No significant differences were noted in blood biochemical values for the experimental animals compared to the unexposed animals as shown in Table III. Long bone radiographs of exposed and control animals revealed no evidence of bone pathology.

Histopathological studies were performed on the control animals as on all exposed animals. These revealed a focal pneumonitis scattered throughout all lobes of the lung of one animal, (see Figure 8). Small nests of encysted nematodes were present, though not necessarily associated with zones of pneumonitis. Histopathological examination of all other organs of these two control animals were considered normal.

Two of the exposed animals revealed presence of encysted pulmonary nematodes and focal microscopic pneumonitis (Figure 9). There was no evidence of atelectasis or emphysema, and the bronchi appeared normal.



Figure 8—Monkey Lung: "Normal" control. Note encysted nematodes (arrows). These organisms and an accompanying focal pneumonitis are endemic in this species.

H&E x 130



Figure 9—Monkey Lung: Animal exposed in 97% He—3%  $\text{O}_2$ , at 200 feet for two weeks and sacrificed immediately after surfacing. Only the endemic nematodes (arrow) with their accompanying pneumonitis are noted.

H&E x 55

In one of the three exposed animals a slight hypertrophy of the zona fasciculata of the adrenal cortex was noted (Figure 10—compare with Figure 11 showing normal adrenal). With these exceptions, all other organs of the three exposed experimental



Figure 10—Monkey Adrenal Gland: Animal exposed in 97% He—3% O<sub>2</sub> at 200 feet for two weeks and sacrificed immediately after surfacing. This hypertrophy of the zona fasciculata was noted in only one of the three exposed monkeys.  
H&E x 50



Figure 11—Monkey Adrenal Gland: This adrenal from a normal control animal may be compared with the case of hypertrophy (Figure 10).  
H&E x 50

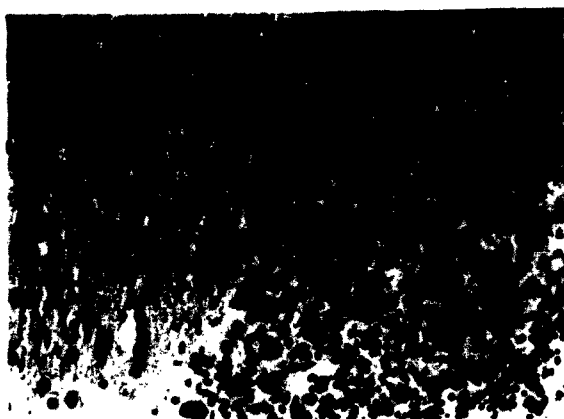


Figure 12—Monkey Cerebellum: Animal exposed in 97% He—3% O<sub>2</sub> at 200 feet for two weeks and sacrificed immediately on surfacing. No abnormalities are noted.

Nissl x 230



Figure 13—Monkey Hippocampus: Same animal as Figure 12. No abnormalities are seen.

Nissl x 50

animals showed no evidence of pathology. Examination of the central and peripheral nervous system in paraffin and celloidin sections revealed no abnormalities in any of these animals (Figure 12, 13, 14, 15). Histological studies aimed at detecting abnormalities in lysosomes, mitochondria, and



Figure 14—Monkey Neocortex: Same animal as Figure 12. No abnormalities are seen.

Nissl x 50



Figure 15—Monkey Cerebral Cortex: Normal control animal.

Nissl x 55

Golgi apparatus of neurons revealed no abnormalities. Recent studies (30, 31, 32) have indicated that alterations in these intracytoplasmic organelles are a useful index of early neuronal necrobiosis. It was considered that the pulmonary pathology seen in the experimental animals was indistinguishable from the inflammatory pulmonary lesions endemic in this species.

Table III—Chronic Exposure of Squirrel Monkeys to an Atmosphere of 97% Helium—3% Oxygen at Seven Atmospheres Pressure for 12 Days

	Blood Studies				
	Exposed Animals			Control Animals	
	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>4</sub>	M <sub>5</sub>
pH	7.39	7.40	7.38	7.40	7.41
CO <sub>2</sub> Content (Vol. %)	46.8	48.3	46.4	47.2	46.8
O <sub>2</sub> Content (Vol. %)	16.9	14.5	16.8	15.5	15.1
(pCO <sub>2</sub> ) p (mm Hg)	41	42	41	41	40
(CO <sub>2</sub> Content) p (Vol. %)	54.7	56.3	54	55.3	56.1
O <sub>2</sub> Capacity (Vol. %)	18.0	17.0	16.3	17.3	18.9
Hemoglobin (gm %)	13.4	12.7	12.9	12.9	14.1
Blood Urea Nitrogen (mgm%)	12.7	15.4	16.0	15.96	18.1
Blood Urea (mgm %)	28.4	33.7	34.1	34.2	35.7
Sugar (mgm %)	97	112	79	86	110
Plasma Sodium (MEq/L)	154	153	156	152	151
Plasma Potassium (MEq/L)	2.8	2.6	2.6	2.7	2.8
Plasma Chloride (MEq/L)	110	120	113	116	123
Carbonic Anhydrase Warburg (Units)	3.61	3.54	3.64	3.6	3.52

## DECOMPRESSION STUDIES ON GOATS

### Experiment No. 1:

Our earlier experiments, as reported here, were concerned with the feasibility of using an artificial atmosphere of oxygen and helium under pressures as great as 200 feet of water. A further problem was to determine decompression requirements in a minimal number of stages after prolonged exposure to severe atmospheric pressures. For this study a series of experiments was performed on goats given saturation exposures of 72 hours in 97% helium—3% oxygen.

### Method:

Two goats, a male weighing 37 lbs. and a female weighing 70 lbs., were exposed to pressure equivalent to that of a 200-foot depth of sea water. This was accomplished in the pressure chamber previously described, by adding helium gas from pressurized cylinders, as in the experiments with albino rats, guinea pigs, and squirrel monkeys.

Carbon dioxide absorption was provided for by spreading 45 lbs. of lithium hydroxide and 24 lbs. of Baralyme pellets in shallow trays with continuous fan circulation of the atmosphere to insure contact with this absorbent material. In addition, 15 lbs. of boric acid powder were added to retard ammonia formation from urea-splitting bacteria acting on excreta. Hay and pulverized commercial



goat feed were provided. Water requirements would be satisfied from an 8-gallon open water container, see Figure 16.



Figure 16—Interior view of compression chamber showing feeding arrangements for goats.

As needed, oxygen was added to the chamber from pressurized bottles to maintain a 3% level ( $160 \pm 10$  mm Hg). The oxygen content of the atmosphere was monitored by a Beckman Model-C Oxygen Analyzer, calibrated in a helium background. Oxygen and  $\text{CO}_2$  content of the chamber atmosphere were also analyzed by Scholander Micro-Gas Analyzer. Ammonia determinations were made by Kitigawa Ammonia Analysis Tubes. Pressure of the atmosphere in the chamber was maintained by addition of helium gas to the chamber from pressurized bottles.

A two-stop 72-hour decompression schedule was followed, (Figure 17).

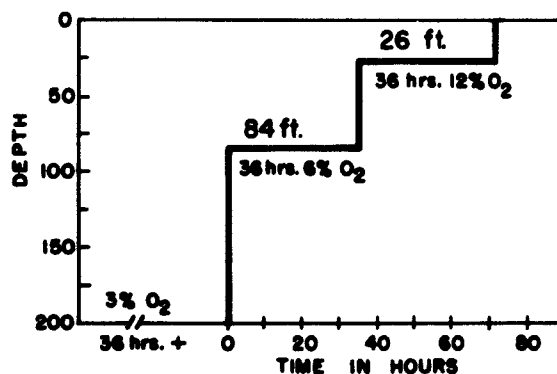


Figure 17—Stages of goats following 72 hours exposure to helium-oxygen atmosphere at 200 feet equivalent depth of sea water.

(1) After keeping the goats for 72 hours at 200 feet, the chamber pressure was reduced to 84 feet in ten minutes. More oxygen was released into the chamber during the ascent so that upon arrival at 84 feet (3.5 atmospheres absolute) the oxygen concentration, measured by the Beckman Analyzer, was raised to 6% (21% effective).

(2) After 36 hours at 84 feet, the chamber pressure was reduced to 26 feet over a period of 30 minutes and oxygen was added during the ascent so that the chamber atmosphere contained 12% oxygen (1.75 atm.) which is 21% effective pressure.

(3) After 36 hours at 26 feet, the chamber was brought to the surface in five minutes.

#### Results:

The two goats fed actively and moved about in the chamber throughout the exposure period and subsequent decompression stages. On the third day, before decompression to 84 feet was begun, Scholander analysis gave 0.358%  $\text{CO}_2$  for an effective concentration of 2.5%. After 30 hours at 84 feet, the  $\text{CO}_2$  analysis on Scholander was 0.14% (0.49% effective). After four hours at 26-foot stop, the ammonia reading by Kitigawa apparatus was 10 ppm. After 11

hours at 26 feet, the CO<sub>2</sub> reading on Scholander was 0.178% (0.311% effective). Ammonia read 30 ppm and CO<sub>2</sub> was 0.24% (0.42% effective) after 34 hours at 26 feet. The animals were observed for 18 hours after surfacing, and showed no evidence of decompression sickness, and none was found during or following decompression to the surface.

#### **Experiment No. 2:**

##### **Method:**

Two goats, both females, weighing 120 lbs. and 35 lbs. respectively, were pressurized to 200 feet in the chamber by addition of helium. Carbon dioxide absorption in this experiment was attempted by means of a scrubber tank containing 15 lbs. of lithium hydroxide in 20 gallons of water, through which the chamber atmosphere was bubbled. A refrigeration-type compressor of 1-CFM capacity removed gas from the chamber, forced it through a diffuser pipe at the bottom of the tank, and then passed the scrubbed gas back into the chamber. Electric fans in the chamber operated continuously to insure atmosphere mixing. Analysis of oxygen and carbon dioxide was accomplished as in the former experiment with goats.

##### **Results:**

Because of insufficient compressor output, difficulty was encountered in CO<sub>2</sub> removal, so that in 17 hours the chamber atmosphere, analyzed by Scholander Micro-Gas Analyzer, was 0.732% (5.1% effective). In spite of continuous scrubber operation and regeneration of solution, the CO<sub>2</sub> percentage rose to 0.9 (6.3% effective) as shown by Kitigawa analysis. A 2-CFM compressor was then substituted and a stainless steel pipe (1½" I.D.) replaced the diffuser pipe which had become sealed over with lithium carbonate deposit. A greater volume of gas scrubbing was achieved, but CO<sub>2</sub> concentration in the chamber was only slowly decreased in the third 24-hour exposure period from 4.5% effective to 2.1% effective.

After 72 hours at 200 feet, the chamber pressure was reduced to 84 feet in 15 minutes. In the next ten minutes, the large goat

became restless, chewed at her left front and left hind legs, and refused to bear weight on the left hind leg. Chamber pressure was reduced to 74 feet in two minutes to determine if signs of decompression sickness were accentuated. These were clearly demonstrated and pressure was promptly increased in the chamber by addition of helium. At 90 feet, the goat again supported herself on all four legs and appeared free of symptoms. The chamber pressure was then returned to the 200-foot level.

A three-stop 72-hour decompression schedule was followed. After three hours at 200 feet, the pressure was reduced to 100 feet over a 20 minute period, and a stop was made at this depth for 24 hours. No recurrence of signs of decompression sickness occurred in either of the two goats during this period. Oxygen concentration was maintained at 21% effective by addition of oxygen to the chamber before and during reduction of pressure. Carbon dioxide remained between 1.6 and 1.8% effective at 100 feet. Following the 24-hour stop at 100 feet, the chamber pressure was reduced to 43 feet in a transition period of 15 minutes. No evidence of decompression sickness occurred in the following 24 hours spent at this stop. Carbon dioxide percentage remained at 1.38 to 1.85% by Kitigawa analysis during this period. The chamber was then brought up to a 10-foot pressure level within ten minutes and this pressure was maintained for 24 hours. No immediate evidence of decompression sickness occurred in the animals on reaching this third stop level. After 10 hours at the 10-foot stop, ammonia concentration by Kitigawa analysis was 30 ppm. After 24 hours at the 10-foot stop, the chamber was brought to the surface in two minutes. No evidence of decompression sickness was observed then or during the following 24-hour period at the surface. The goats weighed 110 lbs. and 38 lbs. respectively, after the six days of exposure to the chamber habitation routine.

#### **Experiment No. 3:**

In our third goat experiment, we intended deliberately to produce decompression sickness in one or both of the experimental

animals. Despite the safety of ratios, (as documented by Hempelmann et al (26)), which were employed in the previous experiment, we could not be completely sure that the unexpected "bends" described in the previous experiment might not have resulted from a close choice in decompression ratio selection, rather than the obvious CO<sub>2</sub> problem. Accordingly, we elected to expose a pair of goats, under ideal atmosphere control, to a much more strenuous decompression regimen. In short, we proposed to decompress these animals quite rapidly from the two hundred foot saturation level to a shallower depth which would ultimately produce decompression sickness, however shallow that depth might be.

As the following history will show, the saturated animals were decompressed on a ratio which is unacceptably dangerous by present applicable standards. Nevertheless, no "bends" resulted. It was decided to make a "stop" at forty feet, since the safety of the 2:1 ratio would here be more than adequately demonstrated, and the experimental animals would be spared the probability of massive decompression sickness and resultant fatality.

#### **Method:**

Two adult female goats weighing 76 and 86 lbs. respectively, with maintenance arrangements as previously described, were pressurized to the 200-foot level in the pressure chamber by addition of helium from pressurized cylinders. This pressure was maintained for 72 hours. Oxygen was maintained at 3% by additions to the chamber atmosphere from a high pressure source. Analyses of oxygen and CO<sub>2</sub> concentrations in the chamber atmosphere were carried out with Beckman Model-C Oxygen Analyzer, Scholander Micro-Gas Analyzer, and Kitigawa CO<sub>2</sub> analysis tubes. Carbon dioxide absorption was accomplished by forcing the chamber atmosphere through two pressure tanks connected in series, each containing 10 lbs. of lithium hydroxide in 20 gallons of water. A refrigeration-type compressor with 2 cu. ft./min. output was used for this purpose. Chamber temperature was to be maintained at 80-89°F by control of room

temperature. Sixteen lbs. of baralyme pellets were spread in shallow trays for auxiliary CO<sub>2</sub> absorption. Two electric fans were operated continuously to insure circulation of the chamber atmosphere.

At the completion of a 72 hour exposure period the chamber pressure was vented off to an equivalent depth of 84 feet in 17 minutes. Oxygen concentration was maintained at 21% effective during ascent and at stops, by addition of oxygen as required from pressurized cylinders. After this rapid change from 7 to 3.5 atmospheres pressure, the goats were observed for a 10 minute period to determine if signs of decompression sickness were evident. The chamber pressure was then reduced to 70 feet in 3 minutes and the animals observed at this depth for 5 minutes. The chamber pressure was further reduced by increments of 10 feet for observation periods of 5 minutes at each step until the 40 foot level was reached. At this depth the animals were observed for signs of decompression sickness for a 24 hour period, after which the chamber pressure was reduced to zero feet in a transition period of 20 minutes and at surface level the animals were observed for a 12 hour period.

#### **Results:**

Both goats were active, moved about the chamber, and fed throughout the exposure period and subsequent stages of decompression. After 18 hours exposure, the CO<sub>2</sub> content of the chamber analyzed 0.246% (1.72% effective) on the Scholander apparatus. After 40 hours exposure CO<sub>2</sub> measured 0.2% (1.4% effective), and after 68 hours exposure 0.04% (0.28% effective). Temperature in the chamber ranged between 86°-87°F. No odor of ammonia was detected in samples of chamber atmosphere during the exposure and subsequent decompression. However, upon surfacing a measurement of 10 ppm was obtained in the chamber by the Kitigawa analyzer.

No evidence of decompression sickness was observed in either animal during reduction of chamber pressure to the 40 foot stop, during the 24 hour stay at this stop,

nor during the 12 hour period of observation at the surface. The animals gained 3 and 2 lbs. in weight respectively during the total experimental period of about 97 hours.

## DISCUSSION AND CONCLUSIONS

Our experiment with rats subjected to normal air under pressure equal to seven atmospheres gave pronounced early deleterious results. These could have been predicted, since previous studies of mammalian survival under conditions of high ambient pressure of air have shown a significant morbidity and mortality. Smith et al, (6), exposed rats to air at four atmospheres absolute for periods up to seventy-two days and found a total morbidity of fifty per cent, resulting from pulmonary edema and hemorrhagic changes. The irritant level for prolonged inhalation of oxygen has been found to be the same for man as for lower animals (428 mm Hg), and 100% oxygen appears to be toxic after a period of inhalation of about 12 hours, (12). Excessive  $pO_2$ , i.e., above 600 mm Hg (6), density of respired gas, and narcotic effect of nitrogen, are all three considered to be factors in the development of pulmonary edema and hemorrhages demonstrated upon autopsy of animals dying within 30 minutes prior to surfacing the chamber.

It remained to be determined whether exposure to a synthetic atmosphere in which oxygen tension is controlled at 160 mm Hg, would permit survival and freedom from pulmonary pathology in animals exposed to the pressure equivalent of 200 feet of sea water. The possibility existed that a density increase equal to seven times that of normal atmospheric air, together with respiratory depression due to the narcotic action of nitrogen, might impair carbon dioxide elimination by decreasing alveolar ventilation sufficiently to be in part responsible for pulmonary tissue changes, (13), (15). Our next experiment gave further information on this problem.

Four guinea pigs and 24 rats (minus one) survived a 14-day exposure to 3% oxygen in nitrogen at seven atmospheres of pres-

sure. Most of the animals lost weight. The rats were not normally active. Focal pulmonary atelectasis and pneumonia were demonstrated in four rats and two guinea pigs which were sacrificed immediately upon completion of the exposure. Sacrifices ten days after surfacing showed one case of focal bronchopneumonia. Although effective oxygen and carbon dioxide concentrations were maintained at values comparable to those in ambient air, the density of the atmosphere was increased by a factor of seven. Increased resistance to airway flow in the lungs, coupled with diminished ventilation due to depression of the respiratory center by high  $pN_2$ , may have been factors in the development of pulmonary atelectasis, (13), (14), (15). Chronic carbon dioxide retention due to decreased pulmonary ventilation was not in evidence, as whole blood pH and  $CO_2$  content were within normal limits upon surfacing. The 24 hour period of gradual decompression may have permitted recovery to near normal values upon surfacing. Maintenance of  $pO_2$  at  $160 \pm 10$  mm Hg would appear to be the important factor in survival of animals when compared to 100% mortality of animals exposed to air at seven atmospheres, as reported in the previous experiment, since atmospheric density and  $pN_2$  would have been comparable. However, it is important that the animals were lethargic and demonstrated intermittent paresis of the hind quarters during this type of exposure.

We found that a relatively nitrogen-free atmosphere containing 21% effective oxygen in helium permitted survival of white rats for 14 days at seven atmospheres pressure. Throughout this period approximately normal activity and feeding were observed. The exposed rats were sacrificed for examination over a period of seven months. These animals had been active; however, they lost

weight, and subsequent to exposure were found to have an average loss of 46 grams. Formation of mold on animal food may have been a significant factor in this development.

Maintenance of  $pO_2$  at  $160 \pm 10$  mm Hg was considered to be an important factor in reduction of pulmonary damage and mortality in this experiment. Since density of the helium-oxygen mixture at seven atmospheres is comparable to air at 1.7 atmospheres absolute, alveolar ventilation was not considered to be significantly affected by the slight density increase. Narcosis resulting in respiratory depression, such as occurs with air breathing at a pressure of seven atmospheres, was avoided by use of helium. Thus, the focal pneumonia and pulmonary atelectasis evident in animals exposed to  $N_2O_2$  in our experiments may have resulted from inadequate alveolar ventilation with a more dense mixture, since density alone was varied with the  $HeO_2$  mixture, the  $pO_2$  being controlled in both experiments at  $160 \pm 10$  mm Hg (13), (15).

As the next step in our study, squirrel monkeys were exposed to the helium-oxygen atmosphere for a prolonged period to determine whether primates were able to maintain normal physiologic functions in an atmosphere which had proved adequate for rats and guinea pigs. The known susceptibility of squirrel monkeys to pulmonary parasites and infections presented a severe test situation. Though histopathological studies revealed encysted pulmonary nematodes and focal pneumonitis in both control and exposed animals, they were normally active and ate well during the exposure period in the compression chamber. Another consideration was the possibility of central nervous system and long bone pathology subsequent to the prolonged exposure and decompression to atmospheric level. However, no abnormalities of these systems were detectable by histopathological and X-ray techniques. The well-known absence of narcotic effects in man from breathing helium-oxygen mixtures at sea levels or in diving, taken into consideration with our experimental results, and more specifically these on monkeys, points to the conclusion that

proper combinations of helium and oxygen may constitute a suitable artificial atmosphere for man at pressures greater than sea level.

Our three consecutive experiments on goats, each lasting six days and involving animals of different sexes, ages, and weights, using two animals at a time, in general, fulfilled expectations in reference to our hypothesis concerning safe decompression requirements.

In the first of this series, a 2:1 ratio of absolute exposure pressure to absolute pressure of the decompression stop, as proposed by Boycott, Damant and Haldane (8), was used to decompress the animals for the first two stops. Decompression to the surface from 26 feet was accomplished on a 1.75:1 ratio. The 72-hour exposure period was considered to be greatly in excess of that required for complete saturation with the helium-oxygen atmosphere to which these animals were exposed. Sutton et al, (9), have reported that five to six hours would appear to be adequate time for saturation to air at the exposure depth. Helium saturation time is reported by Behnke (15) to be somewhat less than air for man. Inasmuch as we were concerned over decompression problems of such "slow" tissues as the bony cortex and the crystalline lens of the eye, we felt justified in prolonging the exposure beyond the saturation levels established in the literature. Elevation of the  $CO_2$  concentration in the chamber atmosphere to 2.5% before decompression commenced was a source of some concern, as increased susceptibility to decompression sickness occurs in the presence of increased carbon dioxide concentration in the inspired atmosphere (25). In this experiment, no evidence of decompression sickness was observed. Uneventful ascent to subsequent stages took place after carbon dioxide concentration of the atmosphere was reduced to less than 0.5% effective.

Stage decompression at depths of 84 and 26 feet, in each of which 36 hours were spent, provided freedom from decompression sickness for two goats previously exposed to

a simulated depth of 200 feet in a chamber while breathing 97% helium—3% oxygen. A 2:1 ratio of absolute exposure depth to absolute depth of the stop was tested for an artificial atmosphere exposure of sufficient duration to allow complete saturation of the test animals.

In the second goat experiment, development of decompression sickness in one animal coming from 200 feet to the 84 foot stop may have been related to the high  $\text{CO}_2$  concentration existing in the previous 24 hours before ascent. This clear-cut case of decompression sickness in an experimental animal offered opportunity to determine whether a saturated subject, developing decompression sickness at a "stop" could safely be treated by return to saturation depth, a "soak" of several hours to permit bubble resolution, and decompression on a more conservative ratio. Accordingly, in this case, recompression to 200 feet for 3 hours was done in an attempt to bring about reduction in size and possible resolution of gas bubbles in tissues producing signs of decompression sickness. Subsequent decompression was carried out on a more conservative 1.75:1 ratio of stops, except for the last stop before surfacing which was 1.3:1. No recurrence of signs of decompression sickness was in evidence.

The use of a 2:1 ratio of exposure pressure to that of decompression stops did not prove adequate to prevent decompression sickness in one of two goats exposed to 97% helium—3% oxygen for 72 hours at

seven atmospheres. However, development of excessive carbon dioxide concentration in the chamber atmosphere may have contributed to this failure. Treatment of the observed decompression sickness in the larger goat was accomplished by returning the animals to previous saturation depth for a three-hour period, following which they were decompressed on a more conservative ratio. In this instance, at least, the described rationale of treatment was effective.

In the third goat experiment it is evident that, for both animals, we safely exceeded the 2:1 ratio. Decompression from 200 feet to 40 feet gives a ratio of absolute pressures of 3.16:1 (231:73), while that from 40 feet to the surface is 2.21:1 (73:33). Approximately one hour elapsed between leaving 200 feet and arriving at 40 feet, during which the animals were observed at decreasing 10 foot levels for evidence of decompression sickness. In this time significant desaturation of well perfused tissues would occur. However, poorly perfused tissues would still contain inert gas tension quite close to that at the exposure depth. Such a state of supersaturation occurring upon reduction of pressure did not cause signs of decompression sickness. The maintenance of relatively low  $\text{CO}_2$  concentration in the chamber may well have been a factor for success in use of a 3.16:1 ratio at depth, when a 2:1 ratio failed to prevent decompression sickness in one animal in our second experiment in which atmosphere  $\text{CO}_2$  was excessive.

## SUMMARY

Using four species of animals, a series of respiration experiments was made in a pressure chamber operated at a level of seven atmospheres. The gases used were: normal air, 97% nitrogen with 3% oxygen; and 97% helium with 3% oxygen. Pressure periods of 72 hours, and of 12 to 14 days, were employed. The requirements for successful decompression were examined. White rats exposed to normal air at the pressure of 200 feet of sea water became lethargic in 15 hours and all were dead in 35 hours. Although the oxygen tension was controlled to  $160 \pm 10$  mm Hg., focal pneumonia and pulmonary atelectasis were demonstrated in albino rats and guinea pigs exposed to the 97% nitrogen—3% oxygen atmosphere. Rats exposed to 97% helium—3% oxygen atmosphere for 14 days were normally active and survived the exposure without significant functional or anatomical changes. The fourfold greater density of the nitrogen-oxygen gas, as compared to the helium-oxygen atmosphere, is considered to be a limiting factor to normal alveolar ventilation, predisposing to the development of pulmonary atelectasis and pneumonia.

Squirrel monkeys were similarly exposed to a synthetic atmosphere of 97% helium—3% oxygen at 200 feet equivalent depth for 14 days. Their blood chemistry and histopathological studies did not differ from those made on our two control monkeys that were breathing atmospheric air and were living

in the unpressurized section of the chamber during the period of the experiment.

Decompression studies carried out on goats exposed to a helium-oxygen atmosphere at 200 feet for 72 hours showed that 36 hour stops at 84 and 26 feet, respectively, were adequate to prevent decompression sickness. One animal was an exception, but accidental excessive carbon dioxide in the atmosphere of that experiment is considered to have facilitated development of decompression sickness. Treatment by return to saturation depth, a short "soak" phase, and decompression on a more conservative schedule proved effective in this case. Subsequent experimentation demonstrated that after 72 hours the pressures could be reduced from 200 to 40 feet, by making short stops with appropriate adjustments of oxygen tension over a period of about one hour. A stay at 40 feet for 24 hours proved adequate to prevent decompression sickness in the two goats similarly exposed.

It may be concluded that a helium-oxygen atmosphere with oxygen tension controlled at  $160 \pm 10$  mm Hg should be satisfactory for 14 days exposure of men at the equivalent pressure of 200 feet of sea water. On the basis of established similarity of decompression requirements for goats and men, decompression stages of 36 hours at 84 and 26 feet, respectively, should provide adequate decompression for saturating exposures at seven atmospheres.

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